If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 20 of page 5 has been amended as follows:

Within still another embodiment the first linker DNA segment encodes ~~is~~ GASAG (SEQ ID NO:29) ~~(SEQ. ID. NO. 29)~~ or GGGGSGGGGSGGGGS (SEQ ID NO:36) ~~(SEQ. ID. NO. 36)~~.

Paragraph beginning at line 23 of page 5 has been amended as follows:

Within yet another embodiment the second linker DNA segment encodes ~~is~~ GGSGG (SEQ ID NO:30) ~~(SEQ. ID. NO. 30)~~ or GGGSGGS (SEQ ID NO:31) ~~(SEQ. ID. NO. 31)~~.

Paragraph beginning at line 29 of page 5 has been amended as follows:

Within another embodiment the peptide is selected from the group consisting of a mammalian GAD 65 peptide, (SEQ ID NO:47) ~~(SEQ ID NO: 59)~~, (SEQ ID NO:89) ~~(SEQ. ID. NO. 61)~~, (SEQ ID NO:40) ~~(SEQ ID NO: 40)~~, (SEQ ID NO:39) ~~(SEQ. ID. NO. 39)~~ and a mammalian myelin basic peptide (SEQ ID NO:33) ~~(SEQ. ID. NO. 33)~~.

Paragraph beginning at line 12 of page 6 has been amended as follows:

Within yet another embodiment the third linker DNA segment encodes ~~is~~ GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:32) ~~(SEQ. ID. NO. 32)~~.

Paragraph beginning at line 1 of page 35 has been amended as follows:

Secretory signals include the α factor signal sequence (prepro sequence: Kurjan and Herskowitz, Cell 30:933-943, 1982; Kurjan et al., U.S. Patent No. 4,546,082; Brake, EP 116, 201), the *PHO5* signal sequence (Beck et al., WO 86/00637) , the *BAR1* secretory signal sequence (MacKay et al., U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the SUC2

signal sequence (Carlsen et al., Molecular and Cellular Biology 3: 439-447, 1983) , the a-l-antitrypsin signal sequence (Kurachi et al., Proc. Natl. Acad. Sci. USA 78: 6826-6830, 1981) , the a-2 plasmin inhibitor signal sequence (Tone et al., J. Biochem. (Tokyo) 102: 1033-1042, 1987) and the tissue plasminogen activator signal sequence (Pennica et al., Nature 301: 214-221, 1983). Alternately, a secretory signal sequence may be synthesized according to the rules established, for example, by von Heinje (European Journal of Biochemistry 133: 17-21, 1983; Journal of Molecular Biology 184: 99-105, 1985; Nucleic Acids Research 14: 4683-4690, 1986). Another signal sequence is the synthetic signal LaC212 spx (1-47) - ERLE (SEQ ID NO:42) described in WO 90/10075.

Paragraph beginning at line 26 of page 47 has been amended as follows:

Plasmid pLJ13 contains the MHC Class II β chain (DRlβ *1501) signal sequence; a myelin basic protein encoding sequence (from bp 283 to 345, encoding amino acids DENPVVHFFKNIVTPRTPPPS 82 to 102) (SEQ. ID. NO. 33); a DNA sequence encoding a flexible linker represented by the amino acid sequence (GGGSGGS SEQ. ID. NO. 31); β1 region of Class II MHC DR1 β*1501 (SEQ ID NOS:120 and 121) ~~(SEQ. ID. NO. 50)~~ encoding sequence: a DNA sequence encoding a flexible linker, represented by the amino acid sequence (GASAG SEQ. ID. NO. 29); and an αl region of Class II MHC DRA*0101 (SEQ ID NOS:90 and 91) ~~(SEQ. ID. NO. 51)~~ encoding sequence. This plasmid was designed to direct secretion of a soluble, fused MHC heterodimer, denoted β1-α1, to which was attached, at the N terminus of β1, a myelin basic protein peptide that has been implicated in multiple sclerosis (Kamholz et al., Proc. Natl. Acad. Sci. USA 83:4962-66, 1986), thus forming a soluble, fused MHC heterodimer:peptide complex.

Paragraph beginning at line 6 of page 48 has been amended as follows:

To construct pLJl3 (SEQ ID NOS:92 and 93) ~~(SEQ. ID. NO. 49)~~ , PCR was used to introduce a DNA sequence encoding MPB at the junction of the signal sequence and β1β2 sequence of the β chain of DRlβ*1501. This was followed by joining the MBP-containing β1 region to the αl region through a linker sequence which was introduced by PCR.

Paragraph beginning at line 27 of page 48 has been amended as follows:

pZCEP was digested with Bam HI and XbaI, and a ~ 0.7 kb SacI-SSP I fragment, comprising the cDNA encoding a chain of DRA*0101, was isolated by agarose gel electrophoresis, and was inserted along with a polylinker sequence containing Bam HI-SacI and SSP I-XbaI ends ~~(SEQ. ID. NO.)~~. The resulting plasmid was designated pSL2.

Paragraph beginning at line 33 of page 48 has been amended as follows:

A cloning site in the linker sequence was generated using PCR by amplifying a ~100 bp Hind III/Cla I fragment containing the signal sequence of Class II b DR1b*1501, to which a sequence encoding the first five amino acids (DPVVH; SEQ ID NO:43) of MBP (82-104) was joined to the 3' end of the signal sequence. The DNA sequence encoding the amino acids VH was chosen to create a unique ApaLI site.

Paragraph beginning at line 10 of page 49 has been amended as follows:

The fragments were digested with Hind Ill/Cla I and Cla I/Xba I, isolated by agarose gel electrophoresis, and inserted into Hind III/Xba I-digested pCZEP. The resulting shuttle plasmid was digested with ApaLI and BamHI, and oligonucleotides encoding the remaining portion of the MBP sequence (represented by the amino acid sequence FFKNIVTPRTPPPS; SEQ ID NO:44) and the start of the flexible linker GGGSG (SEQ ID NO:45) were inserted. The resulting construct contained the MBP sequence joined to the β1β2 sequence of DRIβ*1501 through an intervening linker. The resulting plasmid was designated pSL2l.

Paragraph beginning at line 25 of page 51 has been amended as follows:

A 100 ml PCR reaction was prepared containing 1 ml signal sequence/MBP/linker/β1/linker fragment, 1 ml linker/al fragment, 200 pmol ZC7511 (SEQ. ID. NO. 1), 200 pmol ZC8196 (SEQ. ID. NO. 9), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94 °C for 1 minute, 50 °C for 1 minute, and 72 °C for 1 minute. The 5 amino acid 3' linker (GASAG SEQ. ID. NO. 29) of the signal sequence/MBP/linker/β1/linker fragment overlapped with the same 5 amino acid linker of

the linker/α1 fragment joining the β1 and α1 domains in frame via the 5 amino acid linker. The resulting 730 bp MBP-β1α1 PCR product contained a 5' Hind III site followed by the DR1β*1501 β chain signal sequence, a 21 amino acid MHP peptide DENPVVHFFKNIVTPRTPPPS (SEQ. ID. NO. 33), an 8 amino acid flexible linker (GGGSGGSG; SEQ ID NO:46) attached to the N terminus of the DR1β*1501 β1 domain which was attached to the N terminus of the DRA*0101, α1 domain by a 5 amino acid linker (GASAG SEQ. ID. NO. 29) and ending with a Xba I restriction site. The MBP β1α1 fragment was introduced into Hind III/XbaI pZCEP. A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ13 (human MBP-β1α1).

Paragraph beginning at line 6 of page 54 has been amended as follows:

1) The β1 domain (SEQ ID NOS:94 and 95) ~~(SEQ. ID. NO. 43)~~ of the IA^{g7} NOD mouse β chain was isolated from the β2 domain and fused to linker fragments on both the 5' and 3' ends using PCR.

Paragraph beginning at line 22 of page 54 has been amended as follows:

2) A GAD 65 peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47) ~~(SEQ. ID. NO. 59)~~ and an additional linker fragment were added to the bl/linker fragment from 1 using PCR. In addition, a unique Bam HI site and a the last 16 nucleotides of the phi 10 coupler, adding a second ribosome binding site followed by a stop codon (RBS SEQ. ID. NO. 48) were also added to the 5' end of the GAD peptide to facilitate cloning and expression.

Paragraph beginning at line 30 of page 54 has been amended as follows:

A 100 ml PCR reaction was prepared using 1 ml of eluted bl/linker fragment from above, 200 pmol ZC9473 (SEQ. ID. NO. 15), 200 pmol ZC9479 (SEQ. ID. NO. 17), 200 pmol ZC9480 (SEQ. ID. NO. 18), 10 ml 10X polymerase buffer, 10 ml dNTPs, and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute. The fragments were designed so that all contained overlapping 5' and/or 3' segments, and could both anneal to their complement strand and serve as primers for the reaction. The final 15 3' nucleotides of ZC9499 (SEQ. ID. NO. 23) overlap with the first 15

nucleotides of the β1/linker fragment (ggaggctcaggagga) (SEQ. ID. NO. 35), seamlessly joining the GAD peptide in frame with the β1 domain through a 15 amino acid flexible linker (GGGGSGGGGSGGGGS) (SEQ ID. NO. 36) ZC9479 (SEQ. ID. NO. 17) served as the 5' primer, adding a Bam HI site followed by a RBS (SEQ. ID. NO. 48) to the 5' end of the GAD peptide sequence. A 15 nucleotide overlap (gaggatgattaaatg) (SEQ ID NO:49) between the 3' end of ZC9479 (SEQ. ID. NO. 17) and the first 15 nucleotides of ZC9473 (SEQ. ID. NO. 15) added the sites in frame with the peptide. The resulting 450 bp GAD/β1 fragment was isolated by low melt agarose gel electrophoresis.

Paragraph beginning at line 16 of page 55 has been amended as follows:

3) The αl domain (SEQ ID NOS:96 and 97) ~~(SEQ. ID. NO. 44)~~ of the IA^{g7} was isolated from the 0.2 domain, and fused to a linker fragment on the 5' end and a serine residue, followed by a Spe I and Eco RI site, on the 3' end using PCR.

Paragraph beginning at line 32 of page 55 has been amended as follows:

4) To complete the construct, a final 100 ml PCR reaction was prepared containing 2 ml GAD/β1 fragment from 2), 2 ml α1/linker fragment from 3), 200 pmol ZC9479 (SEQ. ID. NO. 17) , 200 pmol ZC9493 (SEQ. ID. NO. 20) , 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute. The 5 amino acid 3' linker (GGSGG SEQ. ID. NO. 30) of the GAD/βl fragment overlapped with the 5 amino acid linker of the α1/linker fragment joining the β1 and αl domains in frame via the 5 amino acid linker. The resulting GAD-β1α1 PCR product contained a 5' Bam HI site followed by a RBS (SEQ. ID. NO. 48), a 20 amino acid GAD65 peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47) ~~(SEQ. ID. NO.)~~, a 15 amino acid flexible linker (GGGGSGGGGSGGGGS (SEQ. ID. NO. 36) attached to the N terminus of the β1 domain of IA^{g7}, which was attached to the N terminus of the α1 domain of IA^{g7} by a 5 amino acid linker (GGSGG SEQ. IS. NO. 30) and ending with a Spe I and Eco RI restriction site. The GAD-β1α1 fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragments were then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A correct recombinant clone was identified by restriction and sequence analysis and given the designation pLJ18 (GAD-β1α1 IA^{g7}) (SEQ ID NOS:98 and 99) ~~SEQ. ID. NO. 42)~~.

Paragraph beginning at line 23 of page 56 has been amended as follows:

The β1 domain (SEQ ID NOS:100 and 101) ~~(SEQ. ID. NO. 46)~~ of IA^S was isolated from the β2 domain and fused to linker fragments on both the 5' and 3' ends using PCR.

Paragraph beginning at line 31 of page 57 has been amended as follows:

3) The αl domain (SEQ ID NOS:102 and 103) ~~(SEQ. ID. NO. 47)~~ of IA^S was isolated from the α2 domain and fused to a linker fragment on the 5' end, and a serine residue, followed by a Spe I and Eco RI site on the 3' end, using PCR.

Paragraph beginning at line 10 of page 58 has been amended as follows:

4) To complete the construct, a final 100 ml PCR reaction was prepared containing 2 ml MBP/IA^S β1 fragment from 2), 2 ml IA^S αl/linker fragment from 3), 200 pmol ZC9479 (SEQ. ID. NO. 17), 200 pmol ZC9496 (SEQ. ID. NO. 21) 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute. The 5 amino acid 3' linker (GGSGG SEQ. ID. NO. 30) of the MBP/IA^S β1 fragment, overlapped with the same 5 amino acid linker of the IA^S β1/linker fragment, joining the IA^S α1 and IA^S αl domains in frame, via the 5 amino acid linker. The resulting 673 bp MBP-β1α1 IA^S PCR product contained a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP SEQ. ID. NO. 37), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) attached to the N terminus of the IA^S β1 domain, which was attached to the N terminus of the IA^S αl domain by a 5 amino acid linker (GGSGG SEQ ID NO 30), and ending with a Spe I and Eco RI restriction site. The MBP β1α1 fragment was restriction digested with Bam HI and Eco RI, and isolated by low melt agarose gel electrophoresis. The restriction digested fragments were then subcloned onto a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJl9 (MBP β1α1 IA^S) (SEQ ID NOS:104 and 105) ~~SEQ. ID. NO. 45~~).

Paragraph beginning at line 32 of page 59 has been amended as follows:

A 100 ml PCR reaction was prepared containing 100 ng full length lineralized I-A^{g7} β chain (pLJ12), 200 pmol ZC9721 (SEQ. ID. NO. 26), 200 pmol ZC9521 (SEQ. ID. NO. 24), 5 ml 10X polymerase buffer, 5 ml dNTPs and 2.5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. An I-A^{g7} linker/β2 fragment, comprising the β2 domain (SEQ ID NOS:106 and 107) ~~(SEQ. ID. NO. 58)~~, with a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) fused to the 5' end, and stop codon and Eco RI restriction site fused to the 3' end, was obtained. A band of the predicted size was isolated by low melt agarose gel electrophoresis.

Paragraph beginning at line 6 of page 60 has been amended as follows:

3) The α1α2 domain (SEQ ID NOS:108 and 109) ~~(SEQ. ID. NO. 57)~~of the I-A^{g7} was fused to β2 domain of I-A^{g7} using PCR. The 15 amino acid linker sequence on the 3' end of the α1α2 fragment overlapped completely with the same 15 amino acid sequence on the 5' end of the β2 fragment, joining the domains in frame, via a flexible linker.

Paragraph beginning at line 26 of page 60 has been amended as follows:

4) To complete the construct a final 100 ml PCR reaction was prepared containing 5 ml GAD-β1αl fragment from A-4 above, 5 ml I-A^{g7} linker/α1α2/linker/β2 fragment from 3), 200 pmol ZC9521 (SEQ. ID. NO. 24), 200 pmol ZC9479 (SEQ. ID. NO. 17), 10 ml 10X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 30 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes. The entire linker/al portions of both the GAD-β1α1 and linker/α1α2/linker/β2 fragments overlapped, joining the I-A^{g7} β1 and I-A^{g7} α1α2/linker/β2 domains in frame, via the 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30). The resulting GAD-β1α1α2β2 I-A^{g7} PCR product contained a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 20 amino acid GAD peptide (SRLSKVAPVIKARMMEYGTT (SEQ ID NO:47) ~~(SEQ. ID. NO. 59)~~, a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36), attached to the N terminus of the I-A^{g7} β1 domain, which was attached to the N terminus of the α1α2 domain by a 5 amino acid flexible linker (GGSGG, SEQ. ID. NO. 30), and ending with the β2 domain, and an Eco RI restriction site. The GAD-β1 α1α2β2 fragment was restriction digested with Bam HI and Eco RI and

isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ23 (GAD-β1α1α2β2 I-A^{g7}) (SEQ ID NOS:110 and 111) ~~SEQ. ID. NO. 56)~~.

Paragraph beginning at line 1 of page 62 has been amended as follows:

A 100 ml PCR reaction was prepared containing 100 ng full length lineralized IA^S β chain (p40553) , 200 pmol ZC9721 (SEQ. ID. NO. 28), 200 pmol ZC9521 (SEQ. ID. NO. 24) , 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 35 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. An IA^S linker/β2 fragment, comprising the 105 amino acid β2 domain (SEQ ID NOS:112 and 113) ~~(SEQ. ID. NO. 55)~~, with a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36) fused to the 5' end, and stop codon, and Eco RI restriction site, fused to the 3' end, was obtained. A band of the predicted size, 374 bp, was isolated by low melt agarose gel electrophoresis.

Paragraph beginning at line 35 of page 62 has been amended as follows:

4) To complete the construct a final 100 ml PCR reaction was prepared containing 2 ml MBP-β1α1 fragment from B-4 above, 2 ml IA^S linker/α1α2/linker/β2 fragment from 3) , 200 pmol ZC9521 (SEQ. ID. NO. 24), 200 pmol ZC9479 (SEQ. ID. NO. 17), 10 ml l0X polymerase buffer, 10 ml dNTPs and 5 U Taq polymerase. The reaction was carried out for 30 cycles of 94°C for 1 minute, 54°C for 1 minute, and 72°C for 2 minutes. The entire linker/αl portions of both the MBP-β1α1 and linker/ α1α2/linker/β2 fragments overlapped, joining the IA^S β1 and IA^S α1α2/linker/β2 domains, in frame via the 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30). The resulting 1360 bp MBP-β1α1α2β2 IA^S PCR product contained, a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP SEQ. ID. NO. 37), a 15 amino acid flexible linker (GGGGSGGGGSGGGGS SEQ. ID. NO. 36), attached to the N terminus of the IA^S β1 domain, which was attached to the N terminus of the full length IA^S α domain by a 5 amino acid flexible linker (GGSGG SEQ. ID. NO. 30), and ending with the β2 domain and an Eco RI restriction site. The MBP β1α1α2β2 fragment was restriction digested with Bam HI and Eco RI and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI lineralized expression

vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ20 (MBP $\beta 1\alpha 1\alpha 2\beta_2$ IA^S) (SEQ ID NOS:114 and 115) ~~SEQ. ID. NO. 54~~).

Paragraph beginning at line 32 of page 63 has been amended as follows:

1) The $\alpha 1\alpha 2$ domain of the I-A^S (SEQ ID NOS:116 and 117) ~~(SEQ. ID. NO. 53)~~ was fused to a 25 amino acid linker on the 5' end, and a stop codon and Spe I and Eco RI restriction sites on the 3', end using PCR.

Paragraph beginning at line 25 of page 64 has been amended as follows:

There was a 12 amino acid overlap (GGGGSGGGGSGG SEQ. ID. NO. 38) between the 5' end of the 25 amino acid linker, of the linker/$\alpha 1\alpha 2$ fragment, and the 3' end of ZC9499 (SEQ. ID. NO. 23). ZC9499 (SEQ. ID. NO. 23 added a Bam HI restriction site, RBS (SEQ. ID. NO. 48), and MBP peptide(FFKNIVTPRTPPP (SEQ. ID. NO. 37), to the 5' end of the 25 amino acid flexible linker. ZC9479 (SEQ. ID. NO. 17) served as a 5' primer, overlapping the first 32 nucleotides of ZC9499 (SEQ. ID. NO. 23). The resulting 743 bp MBP- $\alpha 1\alpha 2$ IA^S PCR product contained, a 5' Bam HI site, followed by a RBS (SEQ. ID. NO. 48), a 13 amino acid MBP peptide (FFKNIVTPRTPPP (SEQ. ID. NO. 37), a 25 amino acid flexible linker (GGGGSGGGGSGGGGSGGGGSGGGGS SEQ. ID. NO. 32) attached to the N terminus of the IA^S $\alpha 1\alpha 2$ domain, which ended with a Spe I and Eco RI restriction site. The MHP- $\alpha 1\alpha 2$ fragment was restriction digested with Bam HI and Eco RI, and isolated by low melt agarose gel electrophoresis. The restriction digested fragment was then subcloned into a Bam HI-Eco RI lineralized expression vector p27313 (WO 95/11702). A recombinant clone was identified by restriction and sequence analysis and given the designation pLJ21 (MBP-$\alpha 1\alpha 2$ IA^S) (SEQ ID NOS:118 and 119) ~~SEQ. ID. NO. 52~~).

Paragraph beginning at line 34 of page 71 has been amended as follows:

Four individual T cell lines derived from one HLA-DRBl*0404 patient (ThHo) were used to map the 74 synthetic GAD peptides, overlapping sets of 20 mers, that span the entire length of GAD 65 (SEQ ID NO:47) ~~(SEQ. ID. NO. 59)~~. Antigen presenting cells, BLS-DRBl*0404 and/or BLS-DRB1*0401 (Kovats et al, *J. Exp. Med.* 179:2017-22, 1994), were

loaded with peptide by incubating with peptide (about 50 mg/ml) for at least 5 hours. Reactivity of T-cells was determined as above. One peptide, hGAD 33 (PGGAISNMYAMMIARFKNFP SEQ. ID. NO. 40) stimulated 3 or the 4 lines with BLS-Bl*0404. COOH terminal truncations of this peptide from 20 amino acids to an 11 amino acid fragment (PGGAISNMYAM SEQ. ID. NO. 39) when presented by either BLS-B1*0404 or BLS-DRBl*040l, stimulated only one or the T-cell lines. A 10 amino acid fragment (PGGAISNMYA SEQ. ID. NO. 41) stimulated the same T-cell line only when presented by BLS-Bl*0404. This methodology quickly identifies peptide and HLA restriction of T-cell lines and clones as well as identifying GAD epitopes which stimulate T-cell lines derived from a prediabetic donor.

Paragraph beginning at line 20 of page 72 has been amended as follows:

Peptides amidated at the C terminus were synthesized by solid phase peptide synthesis (SPPS) using Fmoc chemistry. Chemicals used in the synthesis were obtained from Nova Biochem (La Jolla, CA) . The peptide was assembled on Rink amide MBHA resin (0.25 millimolar scale) starting from the C terminal end by using a 432A Applied Biosystems, Inc. (Foster City, CA) automated peptide synthesizer and solid phase strategy. The synthesis required double coupling to ensure completion of the coupling reaction, and HBtu-HOBt coupling chemistry was used. Bolded residues required at least double coupling (SRLSKVAPVIKARMMEYGTT-NH2) (SEQ ID NO:50) ~~(SEQ ID NO:59)~~. Each cycle included Fmoc deprotection of amine from the amino acid residue on the resin, and coupling of incoming Fmoc-amino acid. After successful assembly of the peptide, the resin was washed with dichloromethane and dried under vacuum for two hours. The peptide resin was resuspended in 10 ml trifluoroacetic acid (TFA) containing 1 ml of 4-methoxybenzenethiol and 0.7 g of 4-methylmercaptophenol as scavengers. This suspension was gently mixed at room temperature for 2 hours, then filtered through a PTFE filter, and the filtrate was collected in a capped glass bottle containing 1 liter organic solvent mixture (pentane:acetone = 4:1). The white precipitate was allowed to settle at room temperature for 1-2 hours, after which the crude precipitated peptide was isolated by centrifugation. The crude peptide was washed three times with the organic solvent mixture and dried under vacuum overnight.

Paragraph beginning at line 12 of page 76 has been amended as follows:

A series of C-terminal amidated GAD 65 (SEQ ID NO:50) ~~(SEQ. ID. NO. 59)~~ peptides were synthesized where one or more N-terminal or C-terminal amino acids were systematically truncated (Table 3).

Paragraph (Table 3) beginning at line 17 of page 76 has been amended as follows:

--Table 3 Truncated GAD65 peptides from amino acid 524 (1) to amino acid 543 (20). All peptides are amidated at the C-terminus.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SEQ ID NO:
S	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	50
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	51
		L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	52
			S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	53
				K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	54
					V	A	P	V	I	K	A	R	M	M	E	Y	G	T	T	55
						A	P	V	I	K	A	R	M	M	E	Y	G	T	T	56
							P	V	I	K	A	R	M	M	E	Y	G	T	T	57
								V	I	K	A	R	M	M	E	Y	G	T	T	58
									I	K	A	R	M	M	E	Y	G	T	T	59
										K	A	R	M	M	E	Y	G	T	T	60
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G	T		61
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G			62
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y				63
	S	L	S	K	V	A	P	V	I	K	A	R	M	M	E					64
	S	L	S	K	V	A	P	V	I	K	A	R	M	M						65
	S	L	S	K	V	A	P	V	I	K	A	R	M							66
	S	L	S	K	V	A	P	V	I	K	A	R								67
	S	L	S	K	V	A	P	V	I	K	A									68
	S	L	S	K	V	A	P	V	I	K										69
	S	L	S	K	V	A	P	V	I											70

Paragraph (Table 4) beginning at line 1 of page 78 has been amended as follows:

Table 4. Truncated GAD65 core pectides. The C-terminus of each peptide is amidated. 1 is amino acid 524, 20 is amino acid 543.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SEQ ID NO:
			S	K	V	A	P	V	I	K	A	R	M	M	E						71
				K	V	A	P	V	I	K	A	R	M	M	E						72
					V	A	P	V	I	K	A	R	M	M	E						73
						A	P	V	I	K	A	R	M	M	E						74
							P	V	I	K	A	R	M	M	E						75
			S	K	V	A	P	V	I	K	A	R	M	M							76
			S	K	V	A	P	V	I	K	A	R	M								77
			S	K	V	A	P	V	I	K	A	R									78
			S	K	V	A	P	V	I	K	A										79
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G				80
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y					81
	R	L	S	K	V	A	P	V	I	K	A	R	M	M	E						82
		L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G				83
		L	S	K	V	A	P	V	I	K	A	R	M	M	E	Y					84
		L	S	K	V	A	P	V	I	K	A	R	M	M	E						85
			S	K	V	A	P	V	I	K	A	R	M	M	E	Y	G				86
			S	K	V	A	P	V	I	K	A	R	M	M	E	Y					87
			S	K	V	A	P	V	I	K											88

Paragraph beginning at line 40 of page 85 has been amended as follows:

One hundred microliters of the cell-protease inhibitor mixture was added to each well of a 96-well round-bottom plate (Costar, Pleasanton, CA). Fixed NOD cells were co-incubated with biotinylated, C-terminal amidated GAD65 peptide at a concentration of 10,000 nM and unlabeled, Ala scan peptides at concentrations of 100,000, 1,000 and 10 nM for 12-20 hours at 37°C. Mouse serum albumin (MSA), a known allele-specific peptide (SEQ ID NO:89) ~~(SEQ. ID. NO. 61)~~ with high affinity for I-A^{g7}, was used as a positive control, and Eα, which binds to I-A^{d} but not to I-A^{g7}, served as a negative control (Reich et al., J. Immunol. 154: 2279-88, 1994). Following incubation, the plates were vortexed and centrifuged in a Beckman GA-6R centrifuge for 10 minutes at 1500 rpm (Beckman, Fullerton, CA). The supernatant was removed, and the cells were lysed in 60 µl/well of NP-40 lysis buffer (0.5% NP4O, 0.15 M NaCl, 50 mM Tris, pH 8.0, 0.01% sodium azide, and 1:50 dilutions of the protease inhibitor stocks D, E and F (Table 3). The cells were incubated on ice for 30 minutes, with mixing every 15 minutes, followed by centrifuging for 10 minutes at 1500 rpm to obtain a clear lysate.

Paragraph beginning at line 6 of page 91 has been amended as follows:

Newly diabetic NOD mice were irradiated (730 rad) and randomly divided into 4 treatment groups, and splenocytes were isolated as described above. Non-diabetic 7-8 week old, NOD recipient mice were divided into 4 groups. Group one received 1 x 10^7 splenocytes, injected intravenously. Six hours following the injection the mice received a second intravenous injection of either saline, 10 µg/mouse C-terminal amidated GAD65 peptide, or 10, 5, or 1 µg/mouse C-terminal amidated GAD65 peptide-MHC complex. Group two received 2 x 10^7 splenocytes, followed by injections with either saline, 10 µg/mouse C-terminal amidated GAD65 peptide-MHC complex, or 5 µg/mouse MSA-MHC complex. Group three received 1 x 10^7 splenocytes and injections of either saline, 10 µg/mouse C-terminal amidated GAD65 or 200 µg/mouse 10.2.16, an anti-class II antibody. Group four received 1 x 10^7 splenocytes followed by injection with either saline, 20 µg/mouse C-terminal amidated GAD65 peptide, or 1, 5 or 10 µg/mouse C-terminal amidated GAD65 peptide-MHC complex. Group four mice received only two treatments with peptide or peptide-MHC complex, one on day 0 and a second on day 4. All other groups received further treatments on days 8 and 12. The mice were tested for the onset of diabetes by urine analysis. On the day the first animal showed overt signs of diabetes, as

determined by urine and blood glucose levels, mice from each of the treatment groups were randomly selected, and urine and blood glucose levels determined for all selected mice, which were then sacrificed, and spleens and pancreases removed for immunohistochemical analysis. Saline-treated mice developed diabetes within about 12-20 days. Group one mice, which received four treatments of 10 μg peptide-MHC complex, had no significant development of disease by day 30, and did not develop disease until day 75. Those receiving 5 μg peptide-MHC complex had stabilized at 40% diseased mice by day 30, with a gradual increase in disease onset up to day 80, when there was 100% disease among the mice. Those mice in group four, which received only two treatments of peptide-MHC complex, experienced some delayed onset of disease, i.e., less than 50% of those mice receiving 10 μg of peptide-MHC had developed disease by day 30. Blocking with anti-MHC antibody in group three delayed the onset of disease, but provided less protection, i.e., over 75% of those mice receiving 10 μg peptide alone had developed disease by day 30. The C-terminal amidated GAD65 (SEQ ID NO:50) ~~(SEQ. ID. NO. 59)~~ peptide alone accelerated the onset of diabetes in this adoptive transfer model, while the peptide-MHC complex prevented onset of disease.